Rilprivirine and dolutegravir intravaginal rings for the prevention of HIV transmission: *in vitro and in vivo* studies

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Abstract:

Human immunodeficiency virus (HIV) poses a large burden on global health. Through the development of highly active antiretroviral therapy (HAART), HIV infected individuals are living longer lives with undetectable viral loads. In spite of the advancement in treatment, the incidence of new HIV infections continues to increase. Emphasis has now shifted to preventative strategies including intravaginal rings (IVR). The objective of this study was to manufacture IVRs with 10%w/w of rilpivirine (RPV) or dolutegravir (DTG). Based on success of manufacturing, the next steps were *in vitro* and *in vivo* release studies with the goal to have sustained release for a minimum of 30 days. Both *in vitro* release studies surpassed 30 days of zero order release. RPV achieved 100% release and DGT achieved 60% release. *In vivo* mice pharmacokinetics studies were promising since the concentrations of RPV and DTG were above the IC90 locally for the duration of the 28 day study. Plasma concentration of RPV did not reach the IC90 at any point during the study. Overall, RPV and DTG are possible ARTs to be further evaluated for the prevention of HIV using an IVR. Additional studies are needed to determine the efficacy and the possibility of combination IVRs.

Introduction:

Human immunodeficiency virus (HIV) poses a large burden on global health. Worldwide in 2015, 36.7 million people were living with HIV and approximately 2.1 million people were newly infected.[1] Current treatment available for HIV infected individuals consists of combining antiretroviral drugs with differing mechanisms of action, which is often referred to as highly active antiretroviral therapy (HAART). Despite the advancement and success of HAART in decreasing morbidity and morality of individuals with HIV, HAART has been insufficient to curb the pandemic causing a shift to prevention strategies.

Current preventative strategies consist of behavioral interventions, oral antiretroviral (ARV) pre-exposure prophylaxis (PrEP) and topical microbicides. The only FDA approved PrEP therapy is truvada, emtricitabine and tenofovir disoproxil fumarate.[2] Truvada has shown efficacy in prevention of HIV for individuals at high risk, such as men who have sex with men or serodiscordant couples. [3,4,5] Drawbacks to truvada are the importance of adherence and systemic exposure to ARVs in HIV negative individuals. The iPrEx study showed a 44% reduction in HIV infection rate in HIV negative gay men who received daily truvada compared to placebo.[5] In a subanalysis within the iPrEx trial, the adherence rate to the once daily regimen was 51%. The investigators calculated the efficacy would have been at least 92% if the adherence had been 100%.

In order to avoid adherence issues, other routes of administration are available. Sustained delivery to the vaginal tract provides an alternative approach to oral preventative therapy. Benefits of intravaginal delivery are numerous from low systemic exposure, avoiding gastrointestinal and first pass metabolism, and large surface area with a rich blood supply.[6]

Additionally, sterility is not required and patients can easily self-administer. An example of an appropriate delivery device is an intravaginal ring (IVR) because IVRs are designed to deliver drug in a controlled, sustained release fashion. IVRs do not require daily administration thus decreasing the pill burden of the individual. IVRs containing contraceptive and hormone replacement therapy are commercially available and well tolerated.

The objective of the study was to develop and manufacture IVRs containing at least 10% weight by weight ARV. Based on the success of the first objective, the evaluation of the IVRs stability and release *in vivo* would be completed.

Methods:

IVR composition:

The active ARVs used were rilprivirine (RPV), a non-nucleoside reverse transcriptase inhibitor, and Dolutegravir (DTG), an integrase inhibitor. RPV and DTG were chosen because of different mechanisms of action, relevance as viable clinical and commercial products and the lack of pharmacokinetic interactions.[7] The polymer base used for the IVRs was poly(ethylene-co-vinyl acetate) (EVA). EVA is easy to manufacture and hydrophobic in nature which is preferred for sustained release profiles of RPV and DTG. Manufacturing of the IVRs consisted of melting EVA and adding small quantities of active ingredient at a time to achieve uniform dissolution and achieve 10% w/w of active drug. While the polymer was still malleable and warm, a mold was used to form 4mm outer diameter with 1mm cross-section IVR.

In vitro release studies:

IVRs were submerged and fixed in place in one of two buffer solutions, 50% v/v isopropyl alcohol or 25mM sodium acetate (pH 4.2) and 2% w/w solutol. Sodium acetate with solutol at a

pH around 4 simulated the intravaginal environment. The IVRs were submerged at 37°C under sink conditions for a minimum of 30 days. During various time points throughout the 30 days, 1mL samples were taken and buffer volume was replaced. Samples were taken more frequently within the first 24 hours in order to capture any burst release, followed by daily sampling for the first week and once a week thereafter. Drug concentrations were quantified by HPLC.

In vivo pharmacokinetics studies:

BALB/c mice were used as the mouse model for the *in vivo* evaluation of the IVRs (N =3 for each for RPV and DTG). Due to the flexible nature of the mice, the IVRs must be surgically implanted into the mice vaginal cavities. Placebo IVRs consisting of only EVA were produced and used to anchor the active IVR in place (Figure 1). The surgical procedure was modified from Saltzman *et al.*[8] The method had been validated and used for the past three years in the lab prior to this experiment. The animal facility core at University of North Carolina performed the procedure. Plasma and vaginal lavage samples were taken post implantation day 1, 3, 7, 14, 21 and 28 (Figure 2). Where as cervical/vaginal tissue (CVT) samples were taken at day 7, 14 and 28 because the mouse had to be sacrificed in order to obtain the CVT samples.

Figure 1: Drawing of the female mouse reproductive system and the approximate placement of the active IVR and the anchoring placebo IVR.[8]

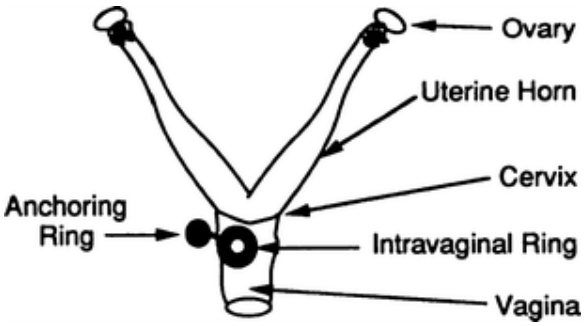
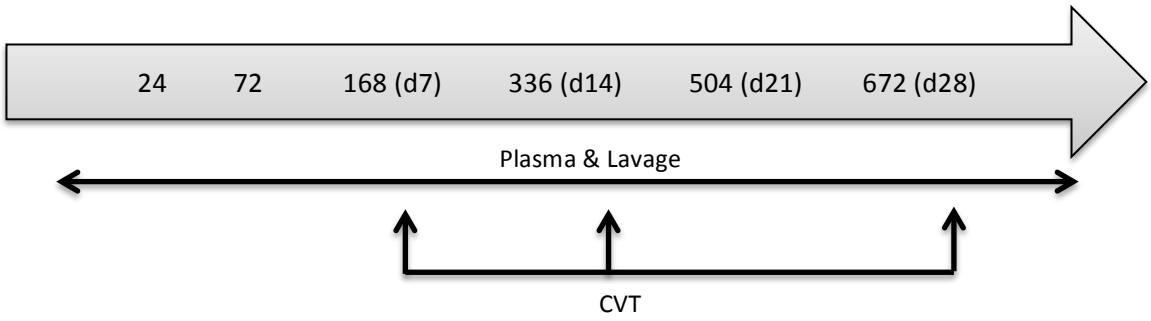


Figure 2: Schematic diagram of sample collection in the BALB/c mice.



Data analysis:

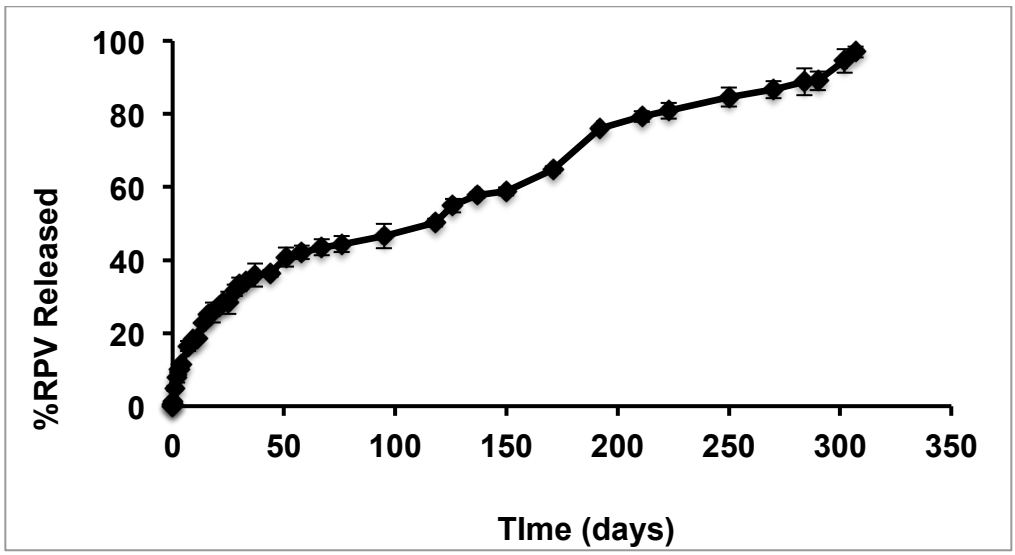
Results:

in vitro

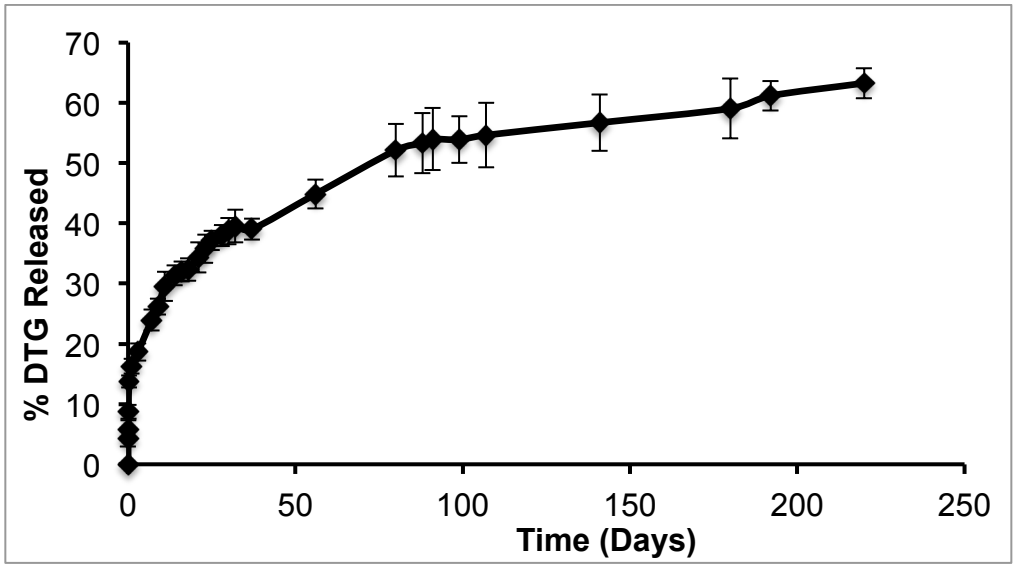
The *in vitro* release studies surpassed the original 30-day requirement. As the release study progressed, both drugs were still being released therefore decided to continue collecting samples. As seen in Figure 3A/3B, RPV achieved a 10% burst release in the first 24 hours with a sustained release of 3.3µg/day. DTG's burst release was about 20% within the first 24 hours with a sustained release of 2.7µg/day. Both reached steady state and maintained zero order kinetics.

Figure 3A/3B:

3A: *in vitro* release of RPV in 50% v/v isopropyl alcohol



3B: *in vitro* release of DTG in 50% v/v isopropyl alcohol



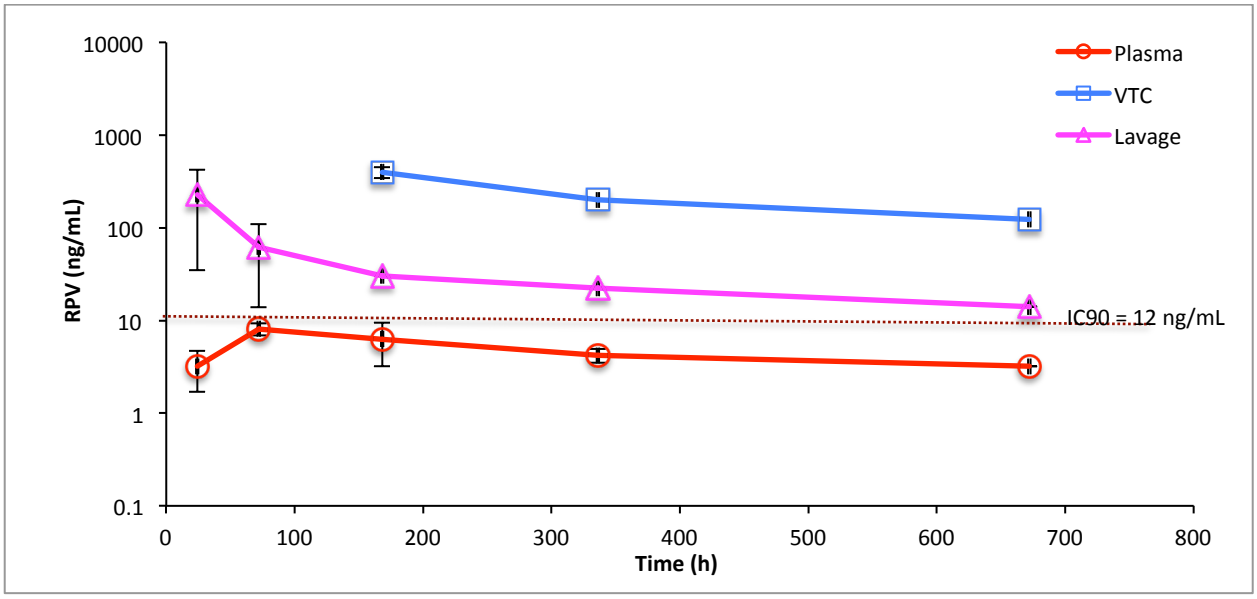
in vivo

Among the three IVRs per active drug used for the *in vivo* study, the amount of DTG ranged from 1.0-1.12mg and RPV ranged from 1.1-1.15mg. The IC90 for RPV and DTG were defined as 12ng/ml and 16.8ng/ml, respectfully. Over the 28 days, mouse vaginal lavage and CVT

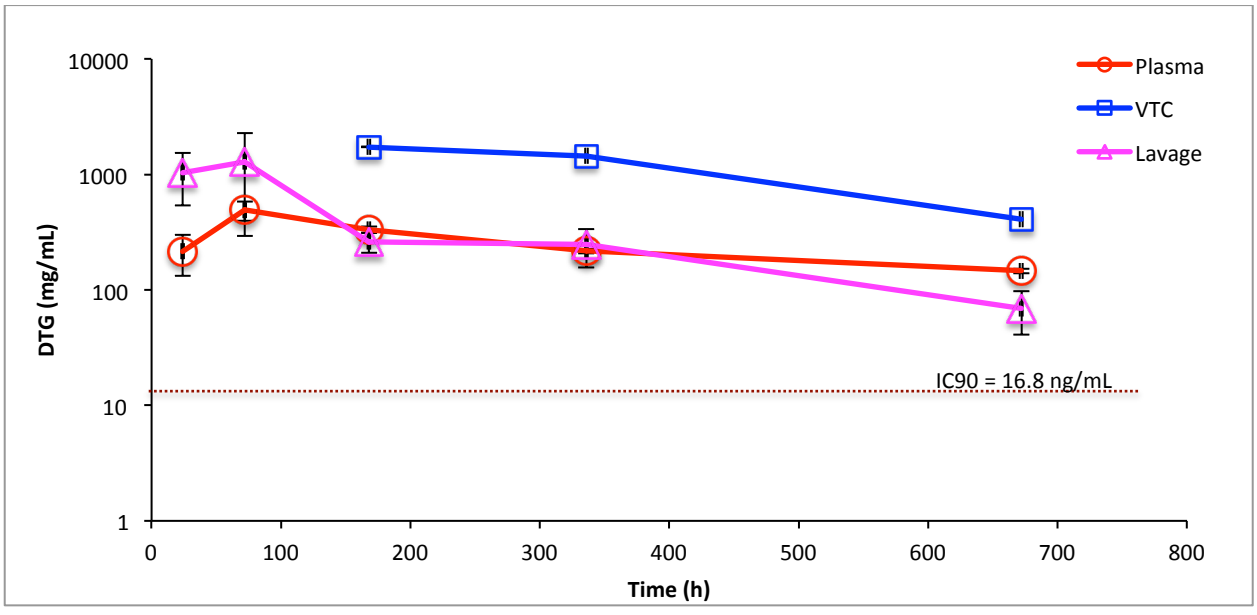
concentrations for both study drugs stayed above the IC90 (Figure 4A/4B). DTG sustained plasma concentrations above the IC90 for the duration of the study. However, RPV plasma concentrations never reached the IC90.

Figure 4A/4B: in vivo release studies

2A. RPV plasma, cervical tissue and vaginal lavage concentrations in BALB/c mice over 28 days.



2B: DTG plasma, cervical tissue and vaginal lavage concentrations in BALB/c mice over 28 days.



Discussion:

During the *in vitro* release studies, RPV rings were manufactured and release study was set up prior to the manufacturing of DTG rings hence why the study is longer than DTG's study. The total amount of RPV was released after about 1 year whereas DTG's release plateaued. A possible reason for the plateau is the loss of sink conditions despite the addition of 1ml buffer following sampling. Another difference is due to release profiles are drug specific. If compared side by side at day 225, 85% of RPV and 60% of DTG had been released from the respected IVRs. Regardless of the differences seen *in intro,* the release profiles of both IVRs were promising and lead to *in vivo* testing.

The evaluation of RPV and DTG containing IVRs *in vivo* was necessary to determine the stability and release profile in the mouse model over an extended period of time. The BALB/c mouse model was chosen due to the cost and availability. Pharmacokinetics is assumed to be consistent throughout mice regardless of immune system function. Both had sustained release of drug for the whole 28 days. Concentration of drug was above the IC90 for both study drugs in the vaginal lavage and vaginal tissue for the study in its entirety. Drug levels locally were high enough to potentially protect against the transmission of HIV vaginally. This is promising for the project moving forward towards the use of combination ART IVRs.

Future studies are needed to evaluate the efficacy of ART IVRs for the prevention of HIV transmission. Next steps include testing efficacy in humanized bone marrow/liver/thymus (BLT) mice. Mice in general are incapable of contracting HIV, therefore a specialized BLT mouse model is necessary. An issue with the current protocol is the invasive procedure needed to implant the IVR. The method involves an abdominal incision and vascular trauma, which in itself

causes an increased risk of contracting HIV when challenged. Therefore an appropriate amount of time must be given for the mouse to heal, which differs from how a female would be using the product.

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